DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

April 8, 2015

INOVA DIAGNOSTICS, INC.
c/o GABRIELLA LAKOS MD, PhD
MEDICAL DIRECTOR, DIRECTOR OF ASSAY DEVELOPEMENT
9900 OLD GROVE ROAD
SAN DIEGO, CA 92131

Re: k150155
Trade/Device Name: NOVA Lite® DAPI ANA Kit
Regulation Number: 21 CFR 866.5100
Regulation Name: Antinuclear antibody immunological test system
Regulatory Class: II
Product Code: DHN, PIV
Dated: January 22, 2015
Received: January 23, 2015

Dear Dr. Lakos:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Leonthena R. Carrington -A

Leonthena R. Carrington, MS, MBA, MT(ASCP)
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K150155

Device Name
NOVA Lite® DAPI ANA Kit

Indications for Use *(Describe)*
NOVA Lite® DAPI ANA Kit is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-nuclear antibodies of the IgG isotype in human serum by manual fluorescence microscopy or with the NOVA View Automated Fluorescence Microscope. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus and other systemic rheumatic diseases. A trained operator must confirm results when generated with the NOVA View device.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

Table of Contents

1. Submitter

Inova Diagnostics, Inc

9900 Old Grove Road,

San Diego, CA, 92131

2. Purpose of submission

New device

3. Devices in the submission

NOVA Lite® DAPI ANA Kit

4. Primary contact

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6. Product Information and Classification

6.1 Proprietary and established names

Proprietary name: NOVA Lite® DAPI ANA Kit

Common name:	Antinuclear antibody kit
Classification name:	antinuclear antibody, indirect immunofluorescent, antigen, control

6.2 Regulatory information

Regulation Description	Antinuclear antibody immunological test system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	DHN
Regulation Number	866.5100
Device Class	II

6.3 Regulatory information

Regulation Description	Automated indirect immunofluorescence microscope and software-assisted system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	PIV
Regulation Number	866.4750
Device Class	II

7. Intended Use

NOVA Lite® DAPI ANA Kit is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-nuclear antibodies of the IgG isotype in human serum by manual fluorescence microscopy or with the NOVA View Automated Fluorescence Microscope. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus and other systemic rheumatic diseases. A trained operator must confirm results when generated with the NOVA View device.

8. Indications for Use

Same as intended use.

9. Predicate device

NOVA Lite HEp-2 ANA Kit, 510k number: k880736

10. Comparison matrix to predicate device

Item	NOVA Lite DAPI ANA Kit	Predicate Device
Intended use	qualitative detection and semi-quantitative determination of anti-nuclear antibodies of IgG isotype in human serum	screening and semi-quantitative determination of anti-nuclear antibodies (ANA) in human serum
Analyte	Anti-nuclear antibodies of IgG isotype	Anti-nuclear antibodies of IgG isotype
Assay methodology	indirect immunofluorescence assay	indirect immunofluorescence assay
Interpretation	by manual fluorescence microscopy or with the NOVA View device	by manual fluorescence microscopy
Antigen	HEp-2 cells substrate, 12-well slides	HEp-2 cells substrate, 12-well slides
Sample type	Serum	Serum
Sample dilution	1:80	1:40
Conjugate	FITC conjugated anti-human IgG (Fc specific) with added 4',6-diamidino-2-phenylindole (DAPI)	FITC conjugated anti-human IgG (Fc specific)
Additional dye in Conjugate	4',6-diamidino-2-phenylindole (DAPI)	None
Controls	Positive (ANA Titratable) and Negative Control	Positive (ANA Titratable) and Negative Control
Storage	2-8 ^{o}C	2-8 ^{o}C
Shelf life	12 months	24 months

11. Type of the product

Assay/reagents including controls.

Qualitative and semi-quantitative.

Device technology: indirect immunofluorescence.

12. Device description

The NOVA Lite DAPI ANA Kit is an indirect immunofluorescence assay for the detection and semi-quantitative determination of anti-nuclear antibodies in human serum.

Kit components:

- HEp-2 (human epithelial cell) substrate slides; 12 wells/slide, with desiccant.
- FITC IgG Conjugate with DAPI, containing 0.09% sodium azide; ready to use.
- Positive Control: ANA Titratable Pattern, human serum with antibodies to HEp-2 nuclei in buffer, containing 0.09% sodium azide; pre-diluted, ready to use.
- Negative Control: IFA System Negative Control, diluted human serum with no ANA present, containing 0.09% sodium azide; pre-diluted, ready to use.
- PBS II (40x) Concentrate, sufficient for making 2000 mL of 1x PBS II.
- Mounting Medium, containing 0.09% sodium azide
- Coverslips

13. Principle of the method and summary of the procedure

Samples are diluted 1:80 in PBS and incubated with the antigen substrate (HEp-2 cells). After incubation, unbound antibodies are washed off. The substrate is then incubated with anti-human IgG-FITC conjugate. The conjugate contains a DNA-binding blue fluorescent dye, 4',6-diamidino-2-phenylindole (DAPI) that is required for NOVA View use. The blue dye is not visible by traditional flurescence microscope at the wavelength where FITC fluorescence is viewed. Unbound reagent is washed off, and the slides are coverslipped. Stained slides are read by manual fluorescence microscopy, or are scanned with the NOVA View Automated Fluorescence Microscope. The resulting digital images are reviewed and interpreted from the computer monitor. ANA positive samples will exhibit an apple green fluorescence corresponding to areas of the cell nuclei where autoantibody has bound. Some sera may contain antibodies reacting with cytoplasmic antigens, and will exhibit apple green fluorescence corresponding to areas of the cell cytoplasm where autoantibody has bound. When slides are assessed by NOVA View, digital images of representative areas of the well are captured. These digital images must be reviewed and interpreted from the computer monitor by a trained operator. Samples that are positive at 1:80 may be titered by performing a 2-fold serial dilution from the initial screening dilution with PBS buffer (i.e. 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, etc.) to determine the endpoint titer.

14. Analytical performance characteristics

15.1 NOVA View Device

The performance characteristics of the NOVA View device are included in a separate submission, *DEN140039*.

15.2. Nomenclature used in the studies

All studies have been performed by interpreting the results with both manual microscopy and with the NOVA View system.

"Manual" and "Manual reading" refers to results obtained by reading the slides with traditional fluorescence microscope.

"NOVA View" refers to raw results obtained with the NOVA View Automated Fluorescence Microscope, such as Light Intensity Units (LIU), positive/negative classification and pattern information.

"Digital", "Digital reading" and "Digital image" refers to results obtained by reading NOVA View generated images on the computer monitor.

If only cytoplasmic pattern is present, the ANA is considered negative.

For statistical calculations, a positive result is presented as "1", and a negative result is presented as "0".

Intensity of the staining is expressed in reactivity grades. Grade 0 is negative; grades 1-4 are weak to strong positive.

When an operator had to read the same set of slides twice (for example, with manual microscope and on the computer monitor), a minimum 3-day "washout" period was included between the readings.

ANA pattern nomenclature:

Patterns reported by NOVA View	
H	Homogeneous
S	Speckled
N	Nucleolar
C	Centromere
D	Dots
U	Unrecognized

15.3 Limit of Blank (LoB), Limit of Detection (LoD)

N/A

15.4 Precision performance

Repeatability and reproducibility

To assess the precision performance of the NOVA Lite DAPI ANA Kit results, a study was performed by processing 3 negative and 10 positive samples ("first set") with various patterns and intensities with NOVA Lite DAPI ANA kit, in three replicates, in 10 runs (2 runs per day), resulting in 30 data points for each sample. The slides were read with NOVA View, and digital images were interpreted by the operator. Slides in this study were not read with manual microscope; i.e. two set of results were generated: NOVA View output and digital image reading results.

Additional precision study ("second set") was performed on 22 samples: 20 negative and around-the cut-off samples, and 2 samples with 3+ average grade intensity level. Samples were tested in three replicates, in 10 runs (2 runs per day), resulting in 30 data points for each sample. The slides were read with NOVA View, and digital images were interpreted, moreover, slides were read with manual microscope, too; i.e. three set of results were generated: NOVA View output, digital image reading results and manual reading results.

A third, separate study has previously been performed ("third set") with samples tested in triplicates or duplicates, in 5 runs, resulting in 15 or 10 data points for each sample. The slides were read with NOVA View, and digital images were interpreted, moreover, slides were read with manual microscope, too; i.e. three set of results were generated: NOVA View output, digital image reading results and manual reading results.

Acceptance criteria: Difference between reactivity grades within one run (between replicates) are within ± one reactivity grade. Average reactivity grade difference between any runs is within ± one reactivity grade.

Results: For both digital image reading and manual reading, grades were within ± one reactivity grade within one run (within triplicates), and the average grade was no more than one reactivity grade different between runs. Pattern was consistent for 100% of the replicates (considering positive results only).

The results of the above three precision studies are summarized in the Table below. Samples in the Table are sorted according to NOVA View LIU values.

			NOVA View output			Manual reading			Digital reading		
	Sample ID	n	Mean LIU	% negative	% positive	Reactivity grade range	% negative	% positive	Reactivity grade range	% negative	% positive
first set	**NVB012**	30	4.7	100	0	N/T	N/T	N/T	0	100	0
	NVB007	30	7.6	100	0	N/T	N/T	N/T	0	100	0
	NVB063	30	7.9	100	0	N/T	N/T	N/T	0	100	0
	NVB111	30	38.5	63.3	36.7	N/T	N/T	N/T	0 - 1	3.3	96.7
	NVB079	30	91.6	13.3	86.7	N/T	N/T	N/T	0 - 1	3.3	96.7
	NVB009	30	229.1	0	100	N/T	N/T	N/T	2 - 3	0	100
	NVB029	30	233.8	0	100	N/T	N/T	N/T	4	0	100
	NVB087	30	310.5	0	100	N/T	N/T	N/T	1 - 2	0	100
	NVB017	30	310.6	0	100	N/T	N/T	N/T	1 - 2	0	100
	NVB023	30	715.5	0	100	N/T	N/T	N/T	4	0	100
	NVB004	30	933.3	0	100	N/T	N/T	N/T	4	0	100
	NVB118	30	1300.1	0	100	N/T	N/T	N/T	4	0	100
	NVB037	30	2217.7	0	100	N/T	N/T	N/T	4	0	100
second set	**NV20**	30	3.5	100	0	0	100	0	0	100	0
	NV16	30	10.2	100	0	0	100	0	0	100	0
	NV2	30	11.4	100	0	0	100	0	0	100	0
	NV8	30	13.5	100	0	0	100	0	0	100	0
	NV15	30	16.6	100	0	0 - 1	13.3	86.7	0 - 1	16.7	83.3
	NV9	30	19.1	100	0	0 - 1	46.7	53.3	0	100	0
	SB24216	30	31.4	86.7	13.3	1	0	100	1	0	100
	NV22	30	33.6	76.7	23.3	0 - 1	96.7	3.3	0 - 1	76.7	23.3
	NV26	30	38.4	90.0	10.0	0 - 1	60.0	40.0	0 - 1	53.3	46.7
	NV14	30	38.8	43.3	56.7	0 - 1	6.7	93.3	1	0	100
	NV13	30	40.5	66.7	33.3	1	0	100	0 - 1	6.7	93.3
	NV5	30	40.7	66.7	33.3	1 - 2	0	100	0 - 1	16.7	83.3
	NVB440	30	43.8	73.3	26.7	0- 1	33.3	66.7	0 - 1	46.7	53.3
	NV4	30	57.5	43.3	56.7	1 - 2	0	100	1 - 2	0	100
	NVB201	30	62.8	26.7	73.3	1 - 2	0	100	1 - 2	0	100
	NVB074	30	63.8	16.7	83.3	1	0	100	1	0	100
	NV12	30	64.8	36.7	63.3	1 - 2	0	100	1 - 2	0	100
	NVB369	30	72.4	23.3	76.7	0- 1	3.3	96.7	0 - 1	13.3	86.7
	NV7	30	74.1	10.0	90.0	1 - 2	0	100	1 - 2	0	100
	NV10	30	128.5	30.0	70.0	1 - 2	0	100	1 - 2	0	100
	NV23	30	822.4	0	100	2 - 3	0	100	3	0	100
	NV6	30	903.9	0	100	3	0	100	3	0	100
third set	**PMDx 5087**	15	24.6	100	0	100	100	0	0	100	0
	SS-A Monospecific 08203	15	103.6	0	100	2	0	100	1 - 2	0	100
	AMA 930328	15	882.6	0	100	4	0	100	4	0	100
	Centromere 120571	10	1052.9	0	100	3 - 4	0	100	4	0	100
	Nucleolar 120559	10	1339.8	0	100	3	0	100	4	0	100
	DNA PS0007 520847	15	1375.6	0	100	3 - 4	0	100	4	0	100
	ANA DNA 420530	10	1607.8	0	100	4	0	100	4	0	100
	SmRNP 220951	10	2811.2	0	100	3	0	100	4	0	100

N/T: Not tested

15.4 Linearity and Analytical Measuring Range (AMR)

N/A

15.5 Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

Interference by bilirubin, hemoglobin, triglycerides, cholesterol and RF IgM was assessed using the following materials and concentrations. The Table below contains all three concentration levels that were tested:

Interfering substance	Manufacturer Part number	Lot number	Concentration #1 tested	Concentration #2 tested	Concentration #3 tested
Bilirubin, conjugated	EMD 201102	DU3038000	10 mg/dL	5 mg/dL	2.5 mg/dL
Hemoglobin	SIGMA H7379	051M7004V	200 mg/dL	100 mg/dL	50 mg/dL
Triglicerydes	SCIPAC P235-8	1201-146	1000 mg/dL	500 mg/dL	250 mg/dL
Cholesterol	SCIPAC P235-8	1201-146	224.3 mg/dL	112.1 mg/mL	56 mg/mL
RF IgM	QC #16	ZG0024783	56 AU	33.6 AU	11.2 AU

Three specimens were tested (one negative, one medium positive, and one strong positive). Interfering substances (hemoglobin, bilirubin, triglycerides, cholesterol) were spiked into every specimen at three different concentrations (see above) in 10% of total specimen volume, and the resulting samples were assessed in triplicates according to the standard protocol (diluted in 1:80 and processed on HEp-2 slides). The concentrations shown above are final (testing) concentrations in the sample after spiking. To assess interference with rheumatoid factor (RF), 10%, 30% and 50% (volume) RF positive sample was added to the test samples. All samples were processed with NOVA Lite DAPI ANA kit, and read with NOVA View. Digital images were interpreted and confirmed. Moreover, all slides were read by the same operator with manual microscopy. Appropriate controls were made by adding 10% (volume) sample diluent to the same samples and for testing for RF interference, adding 10%, 30% and 50% (volume) sample diluent to the samples.

Reactivity grades of samples containing the interfering substance were within ± one grade of the control samples with both manual and digital reading.

No interference was detected with bilirubin up to 10 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 1000 mg/dL, cholesterol up to 224.3 mg/dL and RF IgM up to 56 AU.

15.6 Cross-reactivity

Cross reactivity was examined in the following patient population: patients with autoimmune thyroid disease/TPO antibodies, patients with celiac disease/anti-tTG antibodies, patients with anti-MPO and anti-PR3 antibodies, patients with Crohn's/inflammatory bowel disease, and patients with rheumatoid arthritis.

The number and distribution of the population is shown in the Table below, together with the ANA positivity rate. Considering all 114 samples, the observed positivity rate was 25% for NOVA View results, 27% for digital image reading, and 28% for manual reading. The positivity rate was 15% for NOVA View results, 19% for digital image reading, and 21% for manual reading, when rheumatoid arthritis samples were not included. This positivity rate is in line with the expected results and the published literature. ANA positivity in RA has previously been described with high frequency. RA-33 antibodies are present in up to 36% of RA patients, and anti-histone antibodies have also been identified in the sera of RA patients.

Cross-reactivity cohort, n=114		**Positivity rate**					
		NOVA View		**Manual reading**		**Digital reading**	
Sample type	**Number**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Anti-MPO/anti-PR3	26	1	4%	3	12%	4	15%
Crohn's/Inflammatory bowel disease	20	8	40%	9	45%	8	40%
Autoimmune thyroiditis	24	2	8%	4	17%	3	13%
Celiac disease	24	3	13%	4	17%	3	13%
Rheumatoid arthritis	20	15	75%	11	55%	14	70%
Total	**114**	**29**	**25%**	**31**	**28%**	**32**	**27%**

15.7 Lot to lot comparison

Lot to lot comparison study was performed on three reagent lots. Forty sera were tested along with altogether 8 controls (one Negative and one Positive Control on each slide).

The following comparisons were made:

- NOVA view output: negative/positive, LIU and pattern comparison.
- Digital image reading: negative/positive, grade and pattern comparison
- Manual image reading: negative/positive, grade and pattern comparison.

Agreement:

Average positive, average negative and total agreement between digital image reading result and manual reading results between the three lots were calculated, and the results are shown below:

Digital reading	Lot 008559 vs Lot 009398	Lot 008559 vs Lot 009399	Lot 009398 vs Lot 009399
Average negative agreement	94.7 (85.7-100.0)	91.9 (80.9-100.0)	97.4 (90.9-100.0)
Average positive agreement	95.2 (87.2-100.0)	93.0 (83.3-100.0)	97.6 (91.4-100.0)
Total agreement	95.0 (83.1-99.4)	92.5 (79.6-94.4)	97.5 (86.8-99.9)

Manual reading	Lot 008559 vs Lot 009398	Lot 008559 vs Lot 009399	Lot 009398 vs Lot 009399
Average negative agreement	93.8 (82.8-100.0)	93.8 (82.8-100.0)	100 (100-100)
Average positive agreement	95.8 (88.9-100.0)	95.8 (88.9-100.0)	100 (100-100)
Total agreement	95.0 (83.1-99.4)	95.0 (83.1-99.4)	100 (91.2-100)

Grade agreement:

All grades (100%) were within ± 1 grade from each other for all samples in any pair-wise comparisons with manual and digital reading:

Digital reading grade agreement

	Lot 008559					
Lot 009398	0	1	2	3	4	Total
0	18	2	0	0	0	20
1	0	3	0	0	0	3
2	0	1	2	0	0	3
3	0	0	1	8	0	9
4	0	0	0	0	5	5
Total	18	6	3	8	5	40

	Lot 008559					
Lot 009399	0	1	2	3	4	Total
0	17	2	0	0	0	19
1	1	4	0	0	0	5
2	0	0	2	0	0	2
3	0	0	1	8	0	9
4	0	0	0	0	5	5
Total	18	6	3	8	5	40

	Lot 009398					
Lot 009399	0	1	2	3	4	Total
0	19	0	0	0	0	19
1	1	3	1	0	0	5
2	0	0	2	0	0	2
3	0	0	0	9	0	9
4	0	0	0	0	5	5
Total	20	3	3	9	5	40

Manual reading grade agreement

	Lot 008559					
Lot 009398	0	1	2	3	4	Total
0	15	0	0	0	0	15
1	2	5	0	0	0	7
2	0	1	5	0	0	6
3	0	0	0	6	1	7
4	0	0	0	1	4	5
Total	17	6	5	7	5	40

	Lot 008559					
Lot 009399	0	1	2	3	4	Total
0	15	0	0	0	0	15
1	2	6	0	0	0	8
2	0	0	5	1	0	6
3	0	0	0	5	0	5
4	0	0	0	1	5	6
Total	17	6	5	7	5	40

	Lot 009398					
Lot 009399	0	1	2	3	4	Total
0	15	0	0	0	0	15
1	0	7	1	0	0	8
2	0	0	5	1	0	6
3	0	0	0	5	0	5
4	0	0	0	1	5	6
Total	15	7	6	7	5	40

Pattern agreement:

Pattern agreement was assessed in pair-wise comparison between lots. Only definitive patterns (Homogeneous, Speckled, Centromere, Nucleolar, Nuclear dots) were considered as pattern agreement. User reported "Other" patterns were not considered as an agreement.

There was 100% pattern agreement between the lots with manual and digital image reading, considering positive samples only.

15.8 Accelerated stability study of the anti-human IgG-FITC conjugate with DAPI (P/N 508102)

Accelerated stability study was performed on three lots of conjugate with DAPI according to an isochronous design, at 37 ± 3°C for 4 weeks. Each week a new vial of sealed component was placed in the incubator at 37 ± 3°C, and all components were tested at the end of the study period together with a vial that was stored at 5 ± 3°C (control). Testing was performed by staining the slides with characterized samples. All calculations were performed by comparing results obtained with the control vial (stored at 5 ± 3°C) to those obtained with vials stored at 37 ± 3°C for 1, 2, 3, and 4 weeks, where one week is equal to six months at 5 ± 3°C. All slides were read with NOVA View; moreover, all slides were read by the same operator with manual microscopy.

Acceptance criteria for one year preliminary shelf life:

Reactivity grades obtained on slides stored at 37 °C for 2 weeks are within ± one grade of those obtained on the control slides.

Results are summarized in the table below. Acceptance criteria were met.

	L/N: 042194P1				L/N: 042195P2				L/N: 042196V3			
	Control		Week2		Control		Week2		Control		Week2	
Sample ID	**grade**	**pattern**	**grade**	**pattern**	**grade**	**pattern**	**grade**	**pattern**	**grade**	**pattern**	**grade**	**pattern**
Homogeneous Positive Control	3	H	4	H	3	H	3	H	3	H	3	H
IFA System Negative Control	0	n/a	0	n/a	0	n/a	0	n/a	0	n/a	0	n/a
Speckled Positive Control	3	S	3	S	3	S	4	S	4	S	4	S
Nucleolar Positive Control	3	N	3	N	3	N	3	N	3	N	3	N
Centromere Positive Control	3	C	3	C	3	C	3	C	3	C	3	C
AMA Positive Control	4	D	3	D	3	D	4	D	4	D	3	D
PS0002 420530 (Homogeneous)	1	H	1	H	1	H	1	H	1	H	1	H
PS0005 010047 (Negative)	0	n/a	0	n/a	0	n/a	0	n/a	0	n/a	0	n/a
PS0008 220951 (Speckled)	1	S	0		1	S	1	S	1	S	1	S
PS0006 120559 (Nucleolar)	1	N	1	N	2	N	2	N	2	N	2	N
PS0004 120571 (Centromere	1	C	1	C	1	C	1	C	1	C	1	C
PS0003 930328 (AMA)	2	D	1	D	1	D	1	D	2	D	1	D

16. Cutoff

The serum dilution of 1:80 was selected to provide optimal clinical sensitivity and specificity. The performance of this serum dilution has been validated as described in sections “Clinical performance” and “Expected values”.

17. Comparison with the predicate device

17.1 Conjugate comparison

The NOVA Lite DAPI ANA Kit contains the same components as the predicate device, with the exception of the conjugate. To adapt the assay for use on NOVA View, the blue fluorescent dye DAPI (4',6-diamidino-2-phenylindole) that binds strongly to A-T rich regions in DNA was added to the conjugate. The addition of DAPI does not influence the test utility and performance when used manually, as it is not visible at the wavelength used for reading slides with traditional fluorescence microscope, and does not interfere with antibody binding.

To demonstrate the equivalent performance of the conjugate with and without DAPI, a comparison study has been performed on clinical samples.

407 individual serum samples have been tested. Two sets of slides were stained: one with the conjugate without DAPI (P/N: 508113, included in the predicate device) the other with the conjugate with DAPI (508102). The 1:80 serum dilution was used. The two sets of slides were read by the same operator with an Olympus CX31 fluorescent microscope. Positive/negative agreement, pattern agreement and grade correlation were evaluated.

Acceptance criteria:

Agreement between the two sets is > 85%.

Pattern agreement (for positive samples only) is > 85%.

Grades are within ± one grade from each other for 90% of the samples.

All acceptance criteria were met. Total agreement between the two sets was 96.6%. All grades were within ± one grade from each other. 210 samples were positive with both sets; pattern discrepancy was observed in three cases in those samples that were interpreted as positive with both conjugates.

Agreement:

(N=407)		**508113 (conjugate w/o DAPI)**			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
50102 (conjugate with DAPI)	Negative	183	3	186	Pos. Agreement: 98.6% (95.9-99.7%)
	Positive	11	210	221	Neg. Agreement: 94.3% (90.1-97.1%)
	Total	194	213	407	Total Agreement: 96.6% (94.3-98.1%)

Grade agreement:

	Grades, 508113 (conjugate wo DAPI)					
Grades, 50102 (conjugate with DAPI)	0	1+	2+	3+	4+	Total
0	183	3	0	0	0	186
1+	11	71	8	0	0	90
2+	0	14	73	3	0	90
3+	0	0	2	26	2	30
4+	0	0	0	2	9	11
Total	194	88	83	31	11	407

17.2 Method comparison

Results that were obtained with the NOVA Lite DAPI ANA kit, using 1:80 serum dilution, were compared to those obtained with the reference device (1:40 serum dilution, and anti-human IgG conjugate without DAPI).

The comparison study was performed on 410 samples: 400 clinically characterized sera, and 10 samples with known ANA patterns. All slides were interpreted with traditional fluorescence microscopy only. Interpretation included positive/negative categorization, pattern interpretation and grading of positive samples on a scale of 1+ to 4+.

The distribution of the cohort and the frequency of positive results are shown in the Table below:

	Number of samples	**Number of positive at 1:40**	**Number of positive at 1:80**
Apparently healthy controls	150	41	17
SLE (Systemic Lupus Erythematosus)	100	85	81
SS (Sjogren's syndrome)	30	23	21
SSc (Systemic Sclerosis)	30	20	15
Idiopathic inflammatory myositis (IIM)	10	9	7
MCTD (Mixed Connective Tissue Disease)	20	12	12
Infectious disease	30	6	4
RA (Rheumatoid arthritis)	30	20	17
Centromere antibody positive	5	5	5
Mitochondrial antibody positive	5	4	5
Total	410	224	184

Agreement between the two methods is shown below:

	Predicate device, 1:40		
NOVA Lite DAPI ANA Kit, 1:80	**Negative**	**Positive**	**Total**
Negative	181	45	226
Positive	5	179	184
Total	186	224	410

	Positive Agreement % (95% CI)	**Negative Agreement % (95% CI)**	**Total Agreement % (95% CI)**
1:80 vs 1:40 dilution	79.9 (74.1-85.0)	97.3 (91.5-100.0)	87.7 (84.2-90.8)

Pattern agreement

179 samples were positive according to both dilutions. The number of discrepant samples (not including negative/positive discrepancies, but including patterns interpreted as "other") was five (2.2% of samples that tested positive in 1:40 dilution).

Grade agreement

Fluorescence intensity grades were within ± one grade from each other for 407 samples (99.5%). Grade agreement is shown in the matrix below:

Fluorescence grade NOVA Lite DAPI ANA Kit	**Fluorescence grade, predicate device**					
	0	**1+**	**2+**	**3+**	**4+**	**Total**
0	181	44	1	0	0	**226**
1+	3	35	31	0	0	**69**
2+	1	3	37	24	0	**65**
3+	0	0	0	14	11	**25**
4+	0	0	0	1	23	**24**
Total	**185**	**82**	**69**	**39**	**34**	**409***

**Grade was not reported for one sample*

Out of the 410 samples, 45 samples that were positive in 1:40 dilution were negative in 1:80. Out of the 45 samples, 28 were in from the healthy and infectious diseases population, and 3 had the diagnosis of RA. Fourteen samples were from patients with ANA-associated autoimmune diseases. Two samples

were from patients with IIM, two from patients with Sjogren's syndrome, five had SLE, and five had systemic sclerosis. All these samples had a fluorescence intensity grade of 1+, except for one myositis sample that had a grade of 2+.

The prevalence of ANA in the healthy population (n=150) was 27.3% when sera were tested in 1:40 dilution, and was 11.3% when sera were tested in 1:80 dilution.

Overall clinical sensitivity and specificity is shown below:

	Sensitivity % (95% CI)		**Specificity* %**
	SLE	**SARD**	**(95% CI)**
	(N=100)	**(N=190)**	**(N=60)**
1:40 dilution	85.9 (77.4-92.0)	78.8 (72.3-84.4)	56.7 (43.2-69.4)
1:80 dilution	80.8 (71.7-88.0)	71.4 (64.4-77.8)	65.0 (51.6-76.9)

SARD: Systemic Autoimmune Rheumatic Disease (includes SLE, SSc, SS, MCTD and IIM)

**Control samples include RA and infectious disease population*

18. Agreement between digital reading results and manual reading

18.1 Accuracy of endpoint titration

To assess the accuracy of the system at low analyte levels (around the cut-off), 10 ANA positive samples with various ANA intensities and IIF patterns were titered to endpoint with NOVA Lite DAPI ANA kit at Inova (Site #1) and at two external sites (shown as Site #2 and Site #3) as part of the validation study. The same samples were tested at each site. Samples were diluted in PBS starting at 1:80, and diluted 2-fold until a dilution of 1:40,960 was reached (10 dilutions per sample). All samples were read with NOVA View. Digital images were interpreted and confirmed, and the endpoint titer (the dilution of the last positive result) was determined for each sample. Moreover, all slides were read by the same operator with manual microscopy.

Endpoints by digital reading were the same or within ± 1 dilution steps from that of manual reading for 100%, 60% and 90% of the cases at the three testing sites, and within ± 2 dilution steps for the rest of the samples.

		Site #1				Site #2				Site #3			
		Manual microscopic reading		Digital image reading		Manual microscopic reading		Digital image reading		Manual microscopic reading		Digital image reading	
Number	Sample ID	Pattern	Endpoint titer	Pattern	Endpoint titer	Pattern	Endpoint titer	Pattern	Endpoint titer	Pattern	Endpoint titer	Pattern	Endpoint titer
1	NVB095	C	1280	C	1280	C	640	C	640	C	640	C	320
2	NVB113	C	640	C	640	C	640	C	640	C	320	C	160
3	NVB020	H	640	H	320	H	160	H	160	H	320	H	320
4	NVB071	CS	640	S	640	S	1280	S	320	S	1280	S	1280
5	NVB074	D	320	D	640	D	320	D	640	D	320	D	160
6	NVB056	H	1280	H	640	H	640	H	640	H	1280	H	640
7	NVB042	N	2560	N	2560	N	≥5120	N	1280	N	2560	N	1280
8	NVB014	N	1280	N	1280	N	2560	N	1280	N	1280	N	640
9	NVB118	H	640	H	640	H	1280	H	320	H	1280	H	320
10	NVB036	H	1280	H	1280	H	2560	H	640	H	1280	H	1280

Highlighted results: two dilution steps difference between manual and digital reading.

18.2 Agreement on clinical sample cohort

To assess the agreement between NOVA View generated results, results obtained by reading the digital images and results obtained by manual reading of the slides, a study was performed at Inova Diagnostics (Site #1) and at two external locations (Site #2 and Site #3).

A cohort of 120 samples (same samples at each location) were processed with the NOVA Lite DAPI ANA kit, and read with NOVA View. The 120 samples were selected to represent approximately 50% negative and 50% positive samples with various patterns. All major patterns were represented, and reactivity grades ranged from 0 to 4. Digital images were interpreted and confirmed. Additionally, slides were read with traditional fluorescent microscope by the same operator. Results are presented below.

Agreement between manual reading, digital reading and NOVA View results:

		Positive Agrmnt % (95% CI)	Negative Agrmnt % (95% CI)	Total Agrmnt % (95% CI)
Site #1	Manual vs NOVA View	100.0 (93.7-100.0)	98.4 (91.5-100.0)	99.2 (95.4-100.0)
	Manual vs digital	100.0 (93.7-100.0)	98.4 (91.5-100.0)	99.2 (95.4-100.0)
	Digital vs NOVA View	100.0 (93.8-100.0)	100.0 (94.2-100.0)	100.0 (97.0-100.0)
Site #2	Manual vs NOVA View	95.0 (81.6-99.0)	98.3 (91.1-100.0)	96.7 (91.7-99.1)
	Manual vs digital	96.7 (88.5-99.6)	95.0 (86.1-99.0)	95.8 (90.5-98.6)
	Digital vs NOVA View	93.4 (84.1-98.2)	98.3 (90.9-100.0)	95.8 (90.5-98.6)
Site #3	Manual vs NOVA View	94.6 (85.1-96.8)	98.4 (91.6-100.0)	96.7 (91.7-99.1)
	Manual vs digital	92.9 (82.7-98.0)	100. (94.4-100.0)	96.7 (91.7-99.1)

	Digital vs NOVA View	100.0 (93.2-100.0)	97.1 (89.8-99.6)	98.3 (94.1-99.8)

Pattern agreement

Pattern agreement was assessed in pair-wise comparison between manual reading, NOVA View results, and digital image reading at each site. Only definitive patterns (Homogeneous, Speckled, Centromere, Nucleolar, Nuclear dots) were considered as pattern agreement. NOVA View reported "Unrecognized" patterns and user reported "Other" patterns were not considered as an agreement.

Out of the 120 samples in the reproducibility cohort, there were 57 positive samples at Site#1, 60 at Site#2 and 56 at Site#3 by manual reading (reference method). Summary table of pattern agreement is shown below.

Reproducibility cohort n=120	**Number (%) of samples with pattern agreement***		
	Site#1	**Site#2**	**Site#3**
Manual vs Digital	55 (96.5%)	57 (95.0%)	54 (96.4%)
Manual vs NOVA View	45 (78.9%)	50 (83.3%)	45 (80.4%)
Digital vs NOVA View	44 (77.2%)	48 (80.0%)	45 (80.4%)

**As percentage of samples that were positive with manual interpretation*

Grades correlation(agreement)

Fluorescence intensity grades were within ± one grade from each other between manual reading and digital image reading, as shown below:

Reproducibility cohort n=120	**Percent of samples within ± one grade**		
	Site#1	**Site#2**	**Site#3**
Manual vs digital	98.30%	99.20%	99.20%

19. Clinical performance

19.1 Clinical sensitivity and specificity

To assess clinical performance, a clinical study was performed at Inova Diagnostics (Site #1) and at two external sites (shown as Site #2 and Site #3).

A cohort of 463 clinically characterized samples (same samples at each location) were processed with NOVA Lite DAPI ANA kit, and read with NOVA View. Digital images were interpreted and confirmed. Additionally, slides were read with traditional manual microscope by the same operator.

The number and distribution of the samples are shown below:

Sample type	**Number of samples**
Healthy control	75
HBV	20
HCV	5
HIV	5
Syphilis	5
SLE	75
SSc	20
SS	20
AIL	20
RA	20
MCTD	21
Autoimmune myositis	26
Fibromyalgia	25
Anti-MPO/anti-PR3	26
Crohn's/Inflammatory bowel disease	20
Autoimmune thyroiditis	24
Celiac disease	24
Drug induced lupus	25
Other	7
Total	**463**

Positivity rate in the various sample groups at the three locations is listed below:

Site #1:

		Number of positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	4	4	5
HBV	20	1	5	3
HCV	5	2	0	2
HIV	5	2	0	2
Syphilis	5	3	0	3
SLE	75	60	54	60
SSc	20	19	19	19
SS	20	12	9	13
AIL	20	20	16	20
RA	20	15	11	14
MCTD	21	10	10	10
Autoimmune myositis	26	6	7	6
Fibromyalgia	25	6	9	6
Anti-MPO/anti-PR3	26	1	3	4
Crohn's/Inflammatory bowel disease	20	8	9	8
Autoimmune thyroiditis	24	2	4	3
Celiac disease	24	3	4	3
Drug induced lupus	25	4	5	5
Other	7	1	2	1
Total	**463**	**179**	**171**	**187**

		% positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	5.3%	5.3%	6.7%
HBV	20	5.0%	25.0%	15.0%
HCV	5	40.0%	0.0%	40.0%
HIV	5	40.0%	0.0%	40.0%
Syphilis	5	60.0%	0.0%	60.0%
SLE	75	80.0%	72.0%	80.0%
SSc	20	95.0%	95.0%	95.0%
SS	20	60.0%	45.0%	65.0%
AIL	20	100.0%	80.0%	100.0%
RA	20	75.0%	55.0%	70.0%
MCTD	21	47.6%	47.6%	47.6%
Autoimmune myositis	26	23.1%	26.9%	23.1%
Fibromyalgia	25	24.0%	36.0%	24.0%
Anti-MPO/anti-PR3	26	3.8%	11.5%	15.4%
Crohn's/Inflammatory bowel disease	20	40.0%	45.0%	40.0%
Autoimmune thyroiditis	24	8.3%	16.7%	12.5%
Celiac disease	24	12.5%	16.7%	12.5%
Drug induced lupus	25	16.0%	20.0%	20.0%
Other	7	14.3%	28.6%	14.3%
Total	**463**			

Site #2:

		Number of pos samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	2	7	2
HBV	20	1	4	3
HCV	5	1	1	1
HIV	5	2	0	1
Syphilis	5	1	0	0
SLE	75	54	53	55
SSc	20	19	19	19
SS	20	9	11	9
AIL	20	17	18	17
RA	20	13	15	14
MCTD	21	8	10	8
Autoimmune myositis	26	8	9	7
Fibromyalgia	25	5	11	6
Anti-MPO/anti-PR3	26	5	5	4
Crohn's/Inflammatory bowel disease	20	6	8	7
Autoimmune thyroiditis	24	3	6	2
Celiac disease	24	3	7	5
Drug induced lupus	25	5	5	5
Other	7	1	1	1
Total	**463**	**163**	**190**	**166**

		% positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	2.7%	9.3%	2.7%
HBV	20	5.0%	20.0%	15.0%
HCV	5	20.0%	20.0%	20.0%
HIV	5	40.0%	0.0%	20.0%
Syphilis	5	20.0%	0.0%	0.0%
SLE	75	72.0%	70.7%	73.3%
SSc	20	95.0%	95.0%	95.0%
SS	20	45.0%	55.0%	45.0%
AIL	20	85.0%	90.0%	85.0%
RA	20	65.0%	75.0%	70.0%
MCTD	21	38.1%	47.6%	38.1%
Autoimmune myositis	26	30.8%	34.6%	26.9%
Fibromyalgia	25	20.0%	44.0%	24.0%
Anti-MPO/anti-PR3	26	19.2%	19.2%	15.4%
Crohn's/Inflammatory bowel disease	20	30.0%	40.0%	35.0%
Autoimmune thyroiditis	24	12.5%	25.0%	8.3%
Celiac disease	24	12.5%	29.2%	20.8%
Drug induced lupus	25	20.0%	20.0%	20.0%
Other	7	14.3%	14.3%	14.3%
Total	**463**			

Site #3:

		Number of pos samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	19	13	8
HBV	20	4	2	1
HCV	5	2	2	2
HIV	5	5	2	2
Syphilis	5	3	3	3
SLE	75	62	62	61
SSc	20	19	19	19
SS	20	15	14	14
AIL	20	20	17	18
RA	20	13	15	13
MCTD	21	8	8	8
Autoimmune myositis	26	8	10	7
Fibromyalgia	25	8	9	10
Anti-MPO/anti-PR3	26	5	4	4
Crohn's/Inflammatory bowel disease	20	7	8	7
Autoimmune thyroiditis	24	5	5	4
Celiac disease	24	2	7	4
Drug induced lupus	25	4	7	5
Other	7	2	2	1
Total	**463**	**211**	**209**	**191**

		% positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual read**	**Digital read**
Healthy control	75	25.3%	17.3%	10.7%
HBV	20	20.0%	10.0%	5.0%
HCV	5	40.0%	40.0%	40.0%
HIV	5	100.0%	40.0%	40.0%
Syphilis	5	60.0%	60.0%	60.0%
SLE	75	82.7%	82.7%	81.3%
SSc	20	95.0%	95.0%	95.0%
SS	20	75.0%	70.0%	70.0%
AIL	20	100.0%	85.0%	90.0%
RA	20	65.0%	75.0%	65.0%
MCTD	21	38.1%	38.1%	38.1%
Autoimmune myositis	26	30.8%	38.5%	26.9%
Fibromyalgia	25	32.0%	36.0%	40.0%
Anti-MPO/anti-PR3	26	19.2%	15.4%	15.4%
Crohn's/Inflammatory bowel disease	20	35.0%	40.0%	35.0%
Autoimmune thyroiditis	24	20.8%	20.8%	16.7%
Celiac disease	24	8.3%	29.2%	16.7%
Drug induced lupus	25	16.0%	28.0%	20.0%
Other	7	28.6%	28.6%	14.3%
Total	**463**			

Only four DIL samples were included in the sensitivity calculations, as for the rest of the samples there were concerns about quality of the samples.

Sensitivity was calculated at each site on SLE separately, and on the combination of the systemic autoimmune rheumatic diseases (SARD) (SLE+Sytemic sclerosis+Sjogren's+MCTD+autoimmune myositis+DIL) plus autoimmune liver disease (AIL) population. Specificity was calculated on the total control population excluding healthy subjects. All calculations have been performed according to NOVA View interpretation, digital image reading results and manual (microscopic) reading results. The control population includes samples from patients with RA.

At each testing locations, sensitivity and specificity values had overlapping confidence intervals between NOVA View classification, digital image reading and manual reading, indicating that there are no significant differences between them.

Site #1 (Inova):

	Sensitivity % (95% CI)		**Specificity % (95% CI) (N=174)**
	SLE (N=75)	**SARD+AIL (N=186)**	
NOVA View	80.0 (69.2-88.4)	69.4 (62.2-75.9)	75.3 (68.2-81.5)
Manual reading	72.0 (60.4-81.8)	62.9 (55.5-69.9)	74.1 (67.0-80.5)
Digital reading	80.0 (69.2-88.4)	69.9 (62.8-76.4)	72.4 (65.1-78.9)

Site #2:

	Sensitivity % (95% CI)		**Specificity % (95% CI) (N=174)**
	SLE (N=75)	**SARD+AIL (N=186)**	
NOVA View	72.0 (60.4-81.8)	62.9 (55.5-69.9)	77.0 (70.0-83.0)
Manual reading	70.7 (59.0-80.6)	65.6 (58.3-72.4)	67.2 (59.7-74.2)
Digital reading	73.3 (61.9-82.9)	62.98 (55.5-69.9)	75.3 (68.2-81.5)

Site #3:

	Sensitivity % (95% CI)		**Specificity % (95% CI) (N=174)**
	SLE (N=75)	**SARD+AIL (N=186)**	
NOVA View	82.7 (72.2-90.4)	72.0 (65.0-78.4)	69.0 (61.5-75.7)
Manual reading	82.7 (72.2-90.4)	71.0 (63.9-77.4)	67.2 (59.7-74.2)
Digital reading	81.3 (70.7-89.4)	69.4 (62.2-75.9)	71.3 (63.9-77.9)

Moreover, agreement between NOVA View classification, digital image reading and manual reading were calculated at each testing location, to demonstrate equivalency between results generated by NOVA View, digital image reading (final result of NOVA View reading) and manual reading results. Agreement between digital image reading and manual reading results were greater than 90% at all three testing sites:

Agreement between manual reading, digital image reading and NOVA View results at the three testing sites:

Total agreement between methods % (N=463)	**Site #1**	**Site #2**	**Site #3**
Manual reading vs NOVA View	89.6 (86.5-92.3)	89.8 (86.7-92.4)	87.0 (83.6-90.0)
Manual reading vs digital reading	91.4 (88.4-93.8)	92.2 (89.4-94.5)	92.2 (89.4-94.5)
Digital reading vs NOVA View	97.0 (95.0-98.3)	96.3 (94.2-97.8)	92.2 (89.4-94.5)

Grade agreement

Fluorescence intensity grades as determined by digital image reading were within ± one dilution step from those of determined by traditional manual reading in 96.3%, 99.1% and 99.6% of the samples at the three sites.

Pattern agreement

Pattern agreement was assessed in pair-wise comparison between manual reading, NOVA View results, and digital image reading. Only definitive patterns (homogeneous, speckled, centromere, nucleolar, nuclear dots) were considered as pattern agreement. NOVA View reported "Unrecognized" patterns and user reported "Other" patterns were not considered as an agreement.

Out of the 463 clinical samples, there were 171 positive samples at Site#1, 190 at Site#2 and 209 at Site#3 by manual reading (reference method). Agreement between digital image reading and manual reading was above 90% at all three testing sites.

Summary table of pattern agreement is shown below.

Clinical cohort n=463	**Number (%) of samples with pattern agreement***		
	Site #1	**Site #2**	**Site #3**
Manual vs Digital	162 (94.7%)	174 (91.6%)	200 (95.7%)
Manual vs NOVA View	130 (76.0%)	164 (86.3%)	152 (72.7%)
Digital vs NOVA View	119 (69.6%)	168 (88.4%)	157 (75.1%)

**As percentage of samples that were positive with manual interpretation*

20. Expected values

The clinical validation study population included samples from 75 apparently healthy controls (different from those used for cutoff LIU establishment).

Out of the 75 samples, there were 4, 5 and 4 positive results with manual reading, digital reading, and according to NOVA View classification. The expected result in the normal population is negative; however, 10-20% of positivity may be seen in reference subjects according to published literature and our experience.

21. CDC ANA reference sera

The CDC ANA reference standards (also known as IUIS ANA reference standards) were tested with the NOVA Lite DAPI ANA kit, and read with NOVA View. Digital images were interpreted and confirmed. Additionally, slides were read with traditional manual microscope by the same operator.

All reference sera produced the expected pattern. The results of NOVA View digital image interpretation were within ± one reactivity grade from that of manual interpretation of the slides. No discrepancies in pattern interpretation were seen between manual and digital results.

Results are summarized below:

CDC Reference Serum ID	Expected ANA pattern	Known antibody specificity	Pattern with NOVA Lite DAPI ANA Kit, manual microscopic reading	Pattern with NOVA Lite DAPI ANA Kit, digital image reading
ANA Human Reference Serum #1	Homogeneous/Rim	nDNA	Homogeneous	Homogeneous
ANA Human Reference Serum #2	Speckled	SS-B/La	Speckled	Speckled
ANA Human Reference Serum #3	Speckled	RNP, SS-B/La, SS-A/Ro	Speckled	Speckled
ANA Human Reference Serum #4	Speckled	U1-RNP	Speckled	Speckled
ANA Human Reference Serum #5	Speckled	Sm	Speckled	Speckled
ANA Human Reference Serum #6	Nucleolar	Fibrillarin	Nucleolar	Nucleolar
ANA Human Reference Serum #7	N/A	SS-A/Ro	Speckled	Speckled
ANA Human Reference Serum #8	Centromere	Centromere	Centromere	Centromere
ANA Human Reference Serum #9	N/A	Scl-70	Homogeneous	Homogeneous

ANA Human Reference Serum #10	N/A	Jo-1	ANA Negative; Cytoplasmic speckled (Jo-1 like)	ANA Negative; Cytoplasmic speckled (Jo-1 like)
ANA Human Reference Serum #11	N/A	PM-Scl	Nucleolar	Nucleolar
ANA Human Reference Serum #12	N/A	Ribosomal P	Negative*	Negative*

**Anti-ribosomal antibodies show variable levels of detectability on HEp-2 cells*